SEPARATION OF EPIDERMIS FROM DERMIS BY USE OF DISODIUM CANTHARADIN*

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Recently we have noted that disodium cantharadin can be used effectively in the separation of epidermal cells from the underlying dermis to yield epidermis for immunological or chemical studies. Although the acantholytic action of cantharadin is believed to result from its interference with oxidative carbohydrate metabolism (1), current investigations lead us to believe that cells exposed to very dilute solutions of this reagent, although temporarily altered metabolically, are capable of recovering and functioning in a normal manner.

MATERIALS AND METHODS

1. Disodium cantharadin solution was prepared by dissolving appropriate quantities in sterile saline. After autoclaving at 120° C. for fifteen minutes, these solutions were ready for use. In this manner solutions of molarity ranging from 3×10^{-4} (6 mg/100 cc) to 1.5×10^{-6} (30μg/100 cc) were prepared.

2. Fresh (non-refrigerated) human skin obtained from surgical specimens, removed during the course of simple and radical mastectomies, and rabbit skin obtained from rabbit ears were exposed to sterile cantharadin solution for 2 hours or 18 hours. Following this treatment, the epidermis was readily separated from the dermis by simple peeling. In this manner sheets of epidermal cells were obtained, washed repeatedly with saline, and quick-frozen for subsequent use in other studies.

3. A suspension of *Candida albicans* in saline was exposed to disodium cantharadin in concentrations of 1.5×10^{-4} and 3×10^{-4} M for 2 or 18 hours at 37° C. Subsequently these organisms were regrown or studied for sugar fermentation.

4. Tissue culture cells that had been grown in continuous culture from an operative specimen of serous adenocarcinoma of the ovary obtained in December 1958 were exposed to disodium cantharadin in concentration of 4.5×10^{-4} M (8.5 mg/150 cc). The cells were examined microscopically before and after cantharadin exposure. After incubation at 37° C. with cantharadin in culture media they were transferred to fresh culture media.

RESULTS

1. Epidermis was easily separated from dermis after exposure to disodium cantharadin in concentrations as low as 1.5×10^{-6} M.

2. *Candida albicans* appeared unchanged morphologically after exposure to two hundred times the minimal concentration noted above (3.0×10^{-4} M). These organisms produced normal colonies and chlamydospores when recultured, and metabolized sugars in a normal manner except for the fact that the fermentation of maltose was delayed 48 hours. (The sugars studied included glucose, maltose, lactose, and sucrose.)

3. Tissue culture cells exposed to disodium cantharadin (4.5×10^{-4} M) for two hours appeared unchanged morphologically and were capable of normal reproduction as evidenced by their ability to continue this line of cells when transferred to new media.

COMMENT

From a practical point of view, we have found that disodium cantharadin-induced acantholysis can be used as a rapid and effective method of separating epidermis from dermis. Although previous studies would indicate that cantharadin produces acantholysis through interference with an enzyme or enzyme system, the information we have gathered suggests that disodium cantharadin, when used in extremely low concentrations, does not irreversibly alter the living cell if reproduction and carbohydrate metabolism are used as criteria of normal cellular function. Cantharadin has certain advantages over other methods that have been used to separate epidermis, namely heat, acids, alkalies, and proteolytic enzymes, in that it presumably does not significantly denature epidermal protein. In addition the operations involved in this technic can be carried out under sterile conditions since cantharadin is not inactivated by heating to 120° C. Furthermore cantharadin is not bound to the epidermis as demonstrated in previous investigations (2). The ease of separation of large quantities of epidermal cells makes this method superior to any of the mechanical methods available

In view of the presumed rather specific action of disodium cantharadin (2), it is our belief that epidermal cells obtained in the manner we have described can be used in investigative studies (other than metabolic) in which pure epidermal cells or epidermal protein are required.

REFERENCES

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